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The effects of linseed or chia seed dietary supplementation on adipose tissue development, fatty acid composition, and lipogenic gene expression in lambs

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ABSTRACT

This study examined the effects of linseed or chia seed supplementation on growth and carcass parameters, fatty acid composition, and expression of six lipogenic genes in the intramuscular (IM) and subcutaneous (SC) adipose tissues (AT) in lambs. Thirty-one male *Navarra* breed lambs (56.5 ± 1.6 d old) were weaned at 16.8 ± 0.3 kg live weight (LW) and assigned randomly into three dietary groups: a control group diet of barley and soybean (C); the C diet with 10.5% linseed (L) and the C diet with 10% chia seed (Chia). The lambs were collectively reared in pens (4 weeks) and slaughtered at an average LW of 26.6 ± 0.2 kg. The results indicated that supplementing lambs' diet with either linseed or chia seed, both rich in α-linolenic acid (ALA, C18:3n-3), had no effect on the growth parameters. Supplementation of either linseed or chia seed increased the ALA, C18:1*t*11, eicosapentaenoic acid (EPA, C20:5n-3), and total n-3 fatty acid contents ($P < 0.01$), and decreased the n-6/n-3 ratio in the IM- and SC-AT ($P < 0.001$). Supplementation of linseed increased the proportion of docosapentaenoic acid (DPA; 22:5n-3) in both the IM- and SC-AT ($P < 0.01$), while supplemented chia seed increased the DPA content in the IM-AT ($P < 0.01$). Downregulation of *acetyl-CoA carboxylase 1* or *alpha* (*ACACA*), *stearoyl-CoA desaturase* (*SCD*) and *fatty acid desaturase 2* (*FADS2*) was observed in the IM-AT in the linseed and chia seed-supplemented lambs ($P < 0.05$). The linseed-containing diet did not change *lipoprotein lipase* (*LPL*) or *fatty acid desaturase 1* (*FADS1*) gene expression in the IM-AT, whereas chia seed downregulated the expression of those genes ($P < 0.01$). On the contrary, supplementing either linseed or chia seed had no effect on *ACACA*, *SCD*, or *LPL* gene expression in the SC-AT. Supplementing linseed decreased *FADS1*, *FADS2*, and *fatty acid elongase 5* (*ELOVL5*) mRNA in the SC-AT ($P < 0.01$), whereas supplemented chia seed decreased *ELOVL5* expression ($P < 0.001$). Consequently, changes in fatty acid composition caused by dietary linseed or chia seed might be, at least partly, mediated by the regulation of several genes involved in lipogenesis and the regulation seems to be tissue-specific.

1. Introduction

Animal fat quality is determined by fatty acid (FA) composition, and in recent years several strategies have been tested to improve the nutritional profile of meat to address major public health concerns (Wood et al., 2004). Conjugated linoleic acid (CLA) and n-3 long-chain polyunsaturated fatty acids (LCPUFA) have potential benefits to humans; it would be of interest to increase these types of FA in meat. Different animal feeding trials using seeds rich in n-3 PUFA such as linseed, which has a high α-linolenic acid (ALA) content, have been carried out. Chia (*Salvia*

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hispanica L.), a plant native to southern Mexico and northern Guatemala, is one of the richest botanical sources of ALA and has been rediscovered for animal nutrition (Azcona et al., 2008). Recent research showed that chia seed might be used in the diet of broilers and pigs to increase PUFA concentrations in animal products. Ayerza et al. (2002) reported that including chia seed in the diet of broilers increased the ALA content and lowered the saturated fatty acid (SFA) content in their meat. Furthermore, Coates and Ayerza (2009) showed that the ALA content increased in the subcutaneous (SC) and perirenal (PR) adipose tissues (AT) in pigs when the diet included 20% chia seed. In ruminants, due to the biohydrogenation (BH) of PUFA in the rumen, modifications in the composition of tissue FA, such as ALA and linoleic acid (LA), are limited. Moreover, the effect of dietary chia seed on the FA composition of AT has not yet been studied in sheep.

Linseed supplementation has been shown to influence *de novo* synthesis and monounsaturation of FA in ruminant AT. Corazzin et al. (2013) reported that dietary linseed decreased *stearoyl-CoA desaturase* (*SCD*) gene expression in the SC-AT in bulls. Arana et al. (2010) also reported decreased *SCD* and *acetyl-CoA carboxylase 1* or *alpha* (*ACACA*) gene expression in the intramuscular (IM) and SC-AT in lambs fed a diet containing linseed. Moreover, supplementing linseed was observed to increase the ALA content of the IM-AT, while deposition of LCPUFA, synthesized from ALA, remained unchanged. This was probably due to the inhibitory effect of dietary n-3 PUFA on gene expression of the enzymes involved in LCPUFA biosynthesis.

Since both linseed and chia seed contain high levels of ALA (54% and 64%, respectively) (Azcona et al., 2008), the purpose of this study was to evaluate the effects of linseed and chia seed contained diets on growth and carcass parameters, FA composition, and gene expression of key lipogenic enzymes in the IM- and SC-AT in lambs.

2. Materials and methods

2.1. Animals, diets and sample collection

Thirty-one male *Navarra* breed lambs (56.5 ± 1.3 d old) were weaned at 16.8 ± 0.3 kg live weight (LW) and assigned randomly into three dietary groups: (1) control group (C), concentrate composed of barley and soybean ($n = 9$); (2) linseed group (L), the C concentrate with 10.5% (w/w) linseed supplied by Valomega 160® (Pinallet S.A., Cardona, Spain) consisting of 70% extruded linseed and 30% wheat bran ($n = 11$); (3) Chia group (Chia), the C concentrate with 10% chia seed ($n = 11$) (Table 1). All the diets were isoproteic and isoenergetic. Animal care, handling and experimental procedures were in compliance with the European Union Directive (European Commission, 2010).

After one week of gradual adaptation, the lambs were collectively reared in pens and given *ad libitum* access to concentrate feed and barley straw throughout the experimental period (4 weeks). Lambs were weighed twice weekly in the morning before feeding and slaughtered at a commercial abattoir at 26.6 ± 0.2 kg LW. Immediately after slaughter, carcasses were weighed (HCW) and samples of the IM- (*longissimus dorsi* muscle) and SC-AT were taken for RNA analysis, frozen in N_2, and stored at −80 °C.

Table 1
Measured values of ingredients, chemical composition, and nutrient composition of the C, L, and Chia group diets.

Item	Dietary group[a]		
	C	L	Chia
Ingredient (% as fed)			
Barley	81.2	69.7	73.3
Soybean meal (44% CP)	15.2	11.2	12.6
Linseed (Valomega 160®)[b]	–	15.0	–
Chia seed	–	–	10.0
$CaCO_3$	1.8	1.8	1.8
$NaHCO_3$	1.0	1.0	1.0
NaCl	0.5	0.5	0.5
MVS[c]	0.3	0.3	0.3
Proximate analysis			
Crude protein	16.7	17.0	17.7
Crude fat	3.1	4.8	4.1
Crude fibre	4.0	4.5	5.3
Ash	9.6	8.7	8.9
ME (Mcal/kg)	2.7	2.8	2.8
Fatty acid composition (%)			
C16:0	31.6	16.7	9.8
C18:0	8.9	6.1	3.4
C18:1*c*9	14.9	19.4	7.3
C18:2n-6 (LA)	34.2	20.4	29.9
C18:3n-6	0.03	0.04	0.08
C18:3n-3 (ALA)	4.4	33.7	47.5
C20:0	0.4	0.3	0.3
*c*9,*t*11CLA	0.03	0.02	0.00

ME, metabolic energy; LA, linoleic acid; ALA, α-linolenic acid; CLA, conjugated linoleic acid.

[a] Dietary groups: C = control diet; L = 10.5% linseed supplementation; Chia = 10% chia seed supplementation.

[b] Extruded linseed (Valomega 160®, Pinallet S.A., Cardona, Spain). Product consisted of 70% Tradi-LIN extruded linseed and 30% wheat bran.

[c] MVS: mineral–vitamin supplement. Vitamin A (2,000,000 IU/kg), vitamin D3 (550,000 IU/kg), vitamin E (2000 mg/kg), vitamin B2 (125 mg/kg), vitamin B1 (125 mg/kg), Mg (12,500 mg/kg), Mn (6125 mg/kg), Zn (9900 mg/kg), I (100 mg/kg), Fe (3300 mg/kg), Cu (1100 mg/kg), Co (150 mg/kg), Se (25 mg/kg), anti-rust (62.5 mg/kg).

After chilling for 24 h at 4 °C, carcasses were weighed (cold carcass weight, CCW). Back fat thickness (BFT) was measured in both half-carcasses at a point located 4 cm from the spinal column laterally and 4 cm behind the last rib. All the PR AT was removed and weighed. Samples of IM- and SC-AT were taken and frozen at −20 °C until FA analysis.

2.2. Fatty acid composition

The total lipids in the meat were extracted and hydrolyzed as described by Whittington et al. (1986) with some modifications or optimizations (Aldai et al., 2005).

For FA determination, duplicate 1–2 g muscle tissue and duplicate 0.2–0.3 g subcutaneous adipose tissue were taken. Adipose tissue samples were resuspended in 200 μl of toluene and vortexed for 10 min. Samples were hydrolyzed in 6 ml 5 M KOH in methanol/water (50:50) at 60 °C for 60 min. After dilution with 12 ml of 0.5% NaCl, 5 ml of petroleum spirit was added and vortexed for 5 min, and then 600 μl of absolute ethanol was added and they were centrifuged for 5 min at 800 × *g* and 20 °C. The top layer (containing non-saponifiable extract) was removed